of Raney nickel catalyst. One molar proportion of hydrogen was absorbed in 40 minutes and crystalline Δ^7-compound separated from solution. The mixture was warmed to dissolve the precipitated steroid, filtered and the filtrate concentrated *in vacuo* to dryness. Trituration of the residue with methanol yielded 2.78 g. of practically pure Δ^7-derivative, m.p. 234–237°. After recrystallization from methanol pure Δ^7-isoallospirostene-3β-ol acetate was obtained, m.p. 236–238°, $[\alpha]^{24}D$ −76° ($CHCl_3$).[16]

Anal. Calcd. for $C_{29}H_{44}O_4$: C, 76.27; H, 9.71. Found: C, 76.26; H, 9.57.

Saponification of the acetate yielded Δ^7-spirostene-3β-ol, m.p. 201–204°.

Anal. Calcd. for $C_{27}H_{42}O_3$: C, 78.21; H, 10.21. Found: C, 78.44; H, 10.20.

Preparation of Δ^7-Ergostene-3β-ol.—Two hundred milligrams of Adams catalyst was reduced in 75 cc. of purified ethyl acetate[17] containing 0.1 cc. of glacial acetic acid. Seven hundred ninety-six milligrams of $\Delta^{7,22}$-ergostadiene-3β-ol was added and shaken with hydrogen at 27°. Hydrogen absorption ceased after 50.1 cc. was taken up (theory for 1 mole, 49.5 cc.). After filtration of the mixture and evaporation of the solvent, recrystallization of the residue from methanol yielded 440 mg. of Δ^7-ergostene-3β-ol, m.p. 145–146°, $[\alpha]^{24}D$ +0.44° ($CHCl_3$).[18]

Hydrogenation of $\Delta^{7,9(11),22}$-Ergostatriene-3β-ol Acetate.—A suspension of 200 milligrams of Adams catalyst was reduced in 75 cc. of purified ethyl acetate[17] containing 0.1 cc. of glacial acetic acid. One gram of $\Delta^{7,9(11),22}$-ergostatriene-3β-ol acetate was added and shaken with hydrogen at 27°; hydrogen uptake 98.8 cc. (theory for 2 moles, 97.0 cc.). The solvent was evaporated after removal of the catalyst and the residue recrystallized from methanol–chloroform. The product, Δ^7-ergostene-3β-ol acetate crystallized in plates, m.p. 159–162°, $[\alpha]^{24}D$ −3.1° ($CHCl_3$),[19] end absorption in the ultraviolet region above 220 mμ.

(16) G. Rosenkranz, J. Romo, E. Batres and C. Djerassi, *J. Org. Chem.*, **16**, 298 (1951), reported m.p. 222–223°, $[\alpha]^{20}D$ −66.5° ($CHCl_3$) for Δ^7-isoallospirostene-3β-ol acetate prepared by hydrogenation of the $\Delta^{5,7}$-derivative with platinum in ethyl acetate.

(17) Louis F. Fieser, "Experiments in Organic Chemistry," 2nd Ed., D. C. Heath and Co., Boston, Mass., 1941, p. 364.

(18) A. Windaus and R. Langer report m.p. 145–146°, $[\alpha]D$ +0° ($CHCl_3$).

(19) Previously reported by A. Windaus and R. Langer (ref. 19), m.p. 157° $[\alpha]D$ −5.3° ($CHCl_3$).

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[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF THE UPJOHN COMPANY]

Microbiological Transformations of Steroids.[1] I. Introduction of Oxygen at Carbon-11 of Progesterone

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A microbiological method is described for the oxygenation of steroids at carbon-11 by fungi of the genus *Rhizopus* (order *Mucorales*); yields are high and the oxygenation is accomplished in one simple step. Progesterone yields 11α-hydroxyprogesterone and a dihydroxyprogesterone when oxygenated by *Rhizopus arrhizus* Fischer (A.T.C.C. 11145) or *Rhizopus nigricans* Ehrb. (A.T.C.C. 6227b). The latter species in particular produces excellent yields of 11α-hydroxyprogesterone and in addition, 11α-hydroxyallopregnane-3,20-dione. A simple direct isolation method and characterization of the transformation products are reported.

Discussion

Following the observation[2] that cortisone is effective in the treatment of rheumatoid diseases, a major effort has been devoted to developing an improved synthesis of this hormone. That effort has been necessitated by demand for the drug and by the need to reduce the high cost of producing it.

In the production of cortisone much of the expense is due to the many steps[3–6] which are required to introduce the necessary oxygen atom at carbon-11. Thus a better method of oxygenating at carbon-11 became imperative.

Our objective was to oxygenate readily available steroids directly to the adrenal cortical hormones or to intermediates which could be easily converted to these substances. Accordingly, we were led to investigate the microbiological approach to the oxygenation of steroids because of the manifold and complex reactions which are performed by various microörganisms.

Among the transformations of steroids by microorganisms reported in the literature are the oxidation of a hydroxy group, the reduction of a ketone group, and the reduction of a ring double bond.[7] A conversion of cholesterol to 7-hydroxycholesterol by *Proactinomyces roseus*, was reported by Krámli and Horváth.[8] However, until the publication of our preliminary communication[9] the microbiological oxygenation of steroids at other positions had not been reported. In that article, we outlined a method for the introduction of oxygen into the strategic C-11 position of the steroid nucleus.

In the microbiological transformation of progesterone, as herein described more completely, 11α-hydroxyprogesterone is the main product formed. In addition, small amounts of a dihydroxyproges-

(1) A preliminary report of this work was published as a communication, THIS JOURNAL, **74**, 1871 (1952). See also U. S. Patent 2,602,769 issued July 8, 1952; originally filed Aug. 19, 1950.

(2) P. S. Hench, C. H. Slocumb, A. R. Barnes, H. L. Smith, H. F. Polley and E. C. Kendall, *Proc. Staff Meetings Mayo Clinic*, **24**, 181 (1949); **24**, 277 (1949).

(3) B. F. McKenzie, V. R. Mattox, L. L. Engel and E. C. Kendall, *J. Biol. Chem.*, **173**, 271 (1948).

(4) E. M. Chamberlain, W. V. Ruyle, A. E. Erickson, J. M. Chemerda, L. M. Allminosa, R. L. Erickson, G. E. Sita and Max Tishler, THIS JOURNAL, **73**, 2396 (1951).

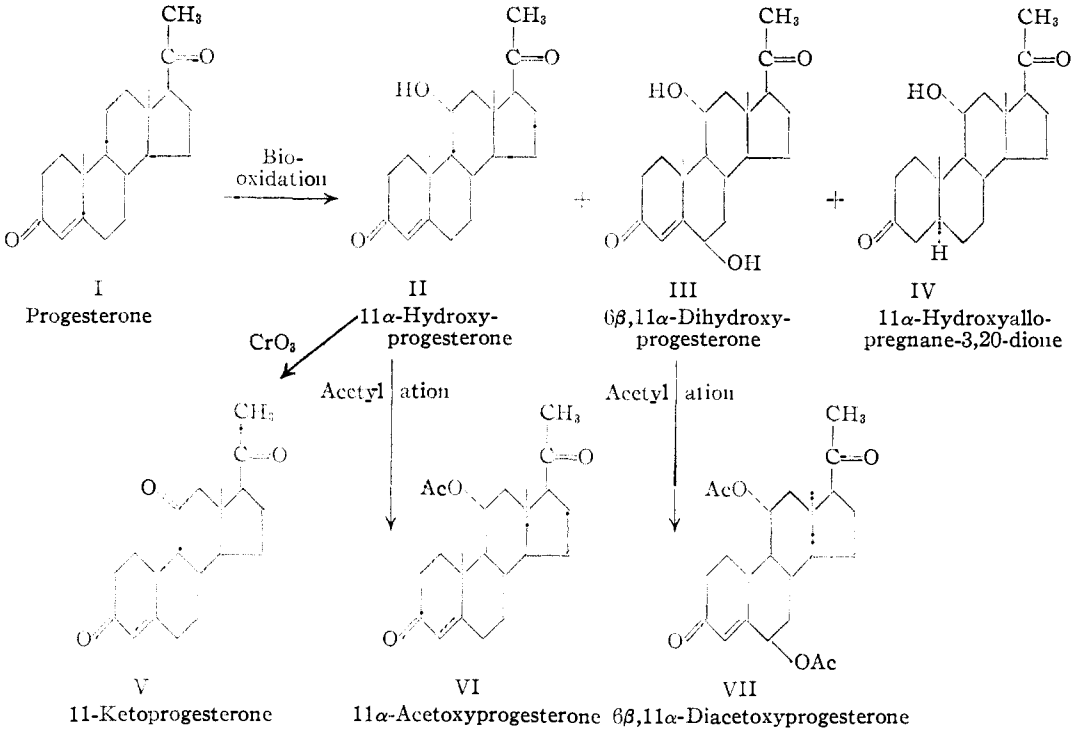
(5) Louis F. Fieser, Josef E. Herz and Wei-Yuan Huang, *ibid.*, **73**, 2397 (1951).

(6) Gilbert Stork, J. Romo, G. Rosenkranz and Carl Djerassi, *ibid.*, **73**, 3546 (1951).

(7) This subject has been reviewed by M. Welsch and G. Heusghem, *Compt. rend. soc. biol.*, **142**, 1074 (1948).

(8) A. Krámli and J. Horváth, *Nature*, **162**, 619 (1948).

(9) Since our preliminary report,[1] Perlman, Titus, and Fried, THIS JOURNAL, **74**, 2126 (1952), have reported the introduction of oxygen at the C-16 position using an unknown Actinomycetes. Colingsworth, Brunner, and Haines, *ibid.*, **74**, 2381 (1952), have also presented evidence for 11-oxygenation of steroids using *Streptomyces fradeae*.



terone and 11α-hydroxyallopregnane-3,20-dione were also isolated.

The fermentations (oxygenation process) were usually carried out by adding the substrate (in this case, progesterone) to a 24-hour growth of the fungus, followed by a 24–48 hour transformation period and the oxygenated products isolated.

In our studies a Rhizopus isolated in this Laboratory and identified as *Rhizopus arrhizus* Fischer (A.T.C.C. 11145) was one of the microörganisms used. With progesterone(I) as the substrate and a 24–48 hour cycle (24-hour growth and 48-hour transformation period) this culture produced two new compounds: a dihydroxyprogesterone (III) in 7% yield and a monohydroxyprogesterone (II) in 10% yield. Eventually yields as high as 50% of II along with 5–15% of III were obtained using this culture.

A comparison of the infrared spectrum and the physical constants of II with those of the known monohydroxylated progesterones[10] indicated that compound II might be 11α-hydroxyprogesterone. Oxidation gave the known 11-ketoprogesterone (V). However, acetylation readily gave a monoacetate under conditions which do not permit the esterification of 11β-hydroxy steroids.[11] The melting point, X-ray diffraction and infrared spectrum of II also differed from those of 11β-hydroxyprogesterone.[10] On the basis of these data II was established as a new compound, 11α-hydroxyprogesterone. The propionate, benzoate and formate of 11α-hydroxyprogesterone are also described.

Based on the elementary analysis and infrared studies of III and its acetate, VII, Compound III was found to contain two hydroxyl groups. A future report will be concerned with this compound, which we have found to be 6β,11α-dihydroxyprogesterone.

In following and isolating the transformation products from the fermentations, the paper chromatography (papergram) technique of Zaffaroni, *et al.*,[12] proved to be very helpful.

Further studies revealed that *Rhizopus nigricans* Ehrb. (A.T.C.C. 6227b) produced high yields (85–95%) of II and only minor amounts of III according to papergram analysis. Isolation work supported the papergram analysis and a simple direct method was devised whereby 80–90% yields of 11α-hydroxyprogesterone were obtained on both small and larger scale conversions (these conversions employed 0.6 g. of progesterone per liter of medium, a 24-hour growth period and a 24-hour transformation period). The 11α-hydroxyprogesterone thus isolated contained 1% or less of unchanged progesterone, 0.5–2.0% of dihydroxyprogesterone, and 0.5–4.0% of 11α-hydroxyallopregnane-3,20-dione (IV). The presence of compound IV, which was later isolated from the mother liquor concentrates of a large scale run, was originally detected in papergram studies by spraying with a solution of 2,4-dinitrophenylhydrazine. The mobility of this compound was identical with that of authentic 11α-hydroxyallopregnane-3,20-dione. Since the progesterone used did not contain allopregnane-3,20-dione, compound IV must therefore be formed from progesterone. The order in which the double bond at carbon-4 is reduced and the carbon at 11 is oxidized is not known.

Using various microörganisms of the order *Mucorales* many other steroids have been oxygenated at carbon-11. Among these are 11-desoxycorticosterone, Reichstein's Compound S, 4-androstene-

(10) C. W. Shoppee and T. Reichstein, *Helv. Chim. Acta*, **24**, 351 (1941); H. G. Fuchs and T. Reichstein, *ibid.*, **23**, 684 (1940).

(11) W. P. Long and T. F. Gallagher, *J. Biol. Chem.*, **162**, 511 (1946); T. F. Gallagher and W. P. Long, *ibid.*, **162**, 521 (1946).

(12) A. Zaffaroni, R. B. Burton and E. H. Keutmann, *Science*, **111**, 6 (1950).

3,17-dione, allopregnane-3,20-dione, pregnane-3,20-dione, allopregnan-3β-hydroxy-20-one, testosterone, 6-dehydroprogesterone, 16-dehydroprogesterone, 3-ketobisnor-4-cholen-22-al and 17α-hydroxyprogesterone. The results of these and other oxygenations will be the subject of future reports.

Experimental

A. General Methods. Fermentation.—Early studies for the purpose of screening common microörganisms used 1–2 mg. of steroidal substrate in 10 ml. of culture media. Later it was found more convenient to use 50–100 mg. per 100 ml. medium in screening studies as well as in larger scale investigations.

Our most commonly used medium (H)[13] was adjusted to a *p*H of 4.3–4.5 with concentrated hydrochloric acid. Twelve liters of this medium in a 5-gal. bottle equipped with paddle and baffle for stirring was sterilized at 120° for 1 hour, and cooled to room temperature. Spores from a week old culture of the desired microörganism (maintained on agar containing 5% malt extract) or a vegetative growth were used for inocula. The incubation temperature was 28° and air was introduced (through a tube extending below the surface) at a rate of approximately 1.0 l. per minute while stirring the medium at about 200 r.p.m. In earlier experiments 4.0 l. of medium in a 5-gal. bottle was agitated on a reciprocal shaker. After a 24-hour incubation period, the steroid, dissolved in acetone or ethanol, was added to the agitated medium; the transformation period proceeded for 24–48 hours. The fermentation was generally complete under these conditions and very little of the substrate remained.

Extraction.—The mycelium was filtered and extracted twice with acetone using an amount approximately equal to the volume of the mycelium; the acetone extracts were added to the filtrate. The mycelium was likewise extracted twice with methylene chloride; these extracts were added to the filtrate and the mycelium was discarded. The combined filtrate and extracts were extracted four times with methylene chloride using each time an amount equal to one-half the volume of the original filtrate, followed by two extractions using each time one-quarter of the volume of the original filtrate. The combined methylene chloride extracts were washed twice with a 2% aqueous solution of sodium bicarbonate, using for each washing an amount equal to one-tenth the volume of the methylene chloride extract and twice with a like amount of water.

The methylene chloride was dried with about 3.5 g. of anhydrous sodium sulfate per liter of solvent and after removal of an aliquot for papergram studies the solvent was removed by distillation. The residue was dissolved in a minimum amount of methylene chloride, filtered into a weighed beaker and the solvent was evaporated by a stream of air at room temperature or by heating on a steam-bath. The crude residue was often crystalline. The transformation products were isolated from this solvent-free concentrate, purified by direct crystallization or chromatography, and characterized.

The steroid transformations were followed by a modified Zaffaroni procedure using Carbitol[14]–methylcyclohexane and propylene glycol–toluene solvent systems; the steroid spots were located by alkaline silver nitrate when appropriate or by the ultraviolet scanner developed in these laboratories.[15]

Direct Crystallization.—When the bioconversions are high, as indicated by papergram analysis, 11α-hydroxyprogesterone (II) can be obtained directly (1) by washing the solvent-free crude crystals with four or five portions of ether (5 ml. per g. of II) or (2) by crystallization from a methylene chloride sirup by addition of ether. If the original methylene chloride sirup is very dark, decolorization can be achieved by treatment with Magnesol[16] (0.25 g. per g. of original steroid). The Magnesol is then washed thoroughly with warm methylene chloride and the washings added to the filtrate. This is concentrated to approximately 2 ml. of methylene chloride per g. of II and ether (10 ml. per g. of II) is added portionwise with scratching (usually not necessary) to induce crystallization, whereupon the remainder of the ether can be added. After one-half hour at room temperature, crystallization is completed at refrigerator temperature in 2 to 3 hours. The crystals are separated by decantation and are washed four times with ether (about 5 ml. per g. of steroid), the ether being decanted after each washing. One such crystallization usually yields a product of high purity representing about 95% of the available product. The ether washings and mother liquor are combined and concentrated, and the process is repeated; sometimes a small second crop of crude material is then obtained. Besides methylene chloride–ether, ethyl acetate or other esters, and methanol or other alcohols, can also be used for crystallization of 11α-hydroxyprogesterone.

B. Progesterone Bioconversion by *Rhizopus arrhizus* Fischer (A.T.C.C. 11145). Dihydroxyprogesterone (III).—To four liters of a 32-hour growth of *Rhizopus arrhizus* was added one gram of progesterone, m.p. 128–129°, $[\alpha]^{23}D$ +198° (chloroform), in 50 ml. of acetone to provide a suspension of the steroid in the medium. After a 48-hour transformation period the *p*H was 3.5. The crude crystalline products which weighed 1.585 g. after isolation as previously described were dissolved in 5 ml. of hot methanol and filtered. After refrigeration overnight the crystals which formed were filtered and washed twice with 2-ml. portions of cold methanol; the yield of III was 75 mg., m.p. 245–249°.[17] Recrystallization from 5 ml. of hot methanol, followed by washing with two 1-ml. portions of cold methanol, yielded 23 mg. of III, m.p. 245–248°, $[\alpha]^{24}D$ +144°, (pyridine). The infrared spectrum, microanalysis and acetylation indicated the addition of two hydroxyl groups to progesterone. The filtrates from all the crystallizations were combined and designated as Fraction A.

Anal. Calcd. for $C_{21}H_{30}O_4$: C, 72.80; H, 8.74. Found: C, 72.83; H, 8.67.

An additional amount (50 mg.) of III was obtained by alumina chromatography.

Diacetoxyprogesterone (VII).—To 9.0 mg. of compound III in a 15-ml. centrifuge tube was added 0.3 ml. of pyridine and 0.3 ml. of acetic anhydride. After allowing the mixture to stand for 16 hours at room temperature, 12.5 ml. of water was added. After 1 hour at room temperature, the mixture was refrigerated for 24–48 hours. The crystals formed were centrifuged, washed with water and dried. The yield of VII was 9.7 mg., m.p. 154–155°. Recrystallization from 0.5 ml. of methanol yielded 6.7 mg. of compound VII, m.p. 153–154°, $[\alpha]^{24}D$ +71° (absolute ethanol). A melting point of 145–148° was also obtained for this diacetate.

Anal. Calcd. for $C_{25}H_{34}O_6$: C, 69.74; H, 7.96. Found: C, 69.80; H, 7.82.

11α-Hydroxyprogesterone (II).—The solids from Fraction A (1.500 g.), after removal of the solvent, were chromatographed over 50 g. of alumina (acid-treated, water-washed, activated at 45° for two hours). The column was eluted using 100-ml. portions of benzene, benzene + 5% ether, benzene + 10% ether, benzene + 50% ether, ether, benzene + 5% chloroform, benzene + 10% chloroform, benzene + 50% chloroform, chloroform, chloroform + 5% acetone, chloroform + 10% acetone, chloroform + 50% acetone, acetone, acetone + 5% methanol, acetone + 10% methanol and acetone + 50% methanol. This procedure resolved the mixture of steroids into more of III and 11α-hydroxyprogesterone (II). The various fractions were combined on the basis of papergram analysis for further purification. The chloroform and chloroform + 5% acetone eluates (414 mg.) were combined and dissolved in 2 ml. of hot methanol and filtered. After overnight refrigeration, 171 mg. of 11α-hydroxyprogesterone was obtained, m.p. 166–167°. Recrystallization from 3 ml. of methanol produced 98.7 mg. of II, m.p. 166–168°, $[\alpha]^{20}D$ +175.9° (chloroform). Microanalysis indicated the addition of one oxygen atom to progesterone.

Anal. Calcd. for $C_{21}H_{30}O_3$: C, 76.32; H, 9.15. Found: C, 76.66; H, 8.92.

(13) Edamin (enzymatic digest of lactalbumin obtained from Sheffield Farms, New York, N. Y.), 20 g.; corn steep, 3.0 g.; technical dextrose, 50 g.; tap water, *q.s.* 1.0 l.

(14) Carbitol is diethylene glycol monoethyl ether obtained from Carbide and Carbon Chemicals Division, New York, N. Y.

(15) W. J. Haines and N. Drake, *Federation Proc.*, **9**, 180 (1950).

(16) An adsorptive magnesium silicate obtained from Westvaco Chemical Division, 405 Lexington Avenue, New York, N. Y.

(17) All melting points were taken on a Fisher–Johns block and are uncorrected unless otherwise specified.

Infrared studies on II indicated the introduction of hydroxyl into the progesterone molecule.

11-Ketoprogesterone (V).—To 30 mg. of 11α-hydroxyprogesterone (II) in 0.5 ml. of glacial acetic acid was added a solution of 5 mg. of chromium trioxide in 0.005 ml. of water and 1 ml. of glacial acetic acid. The mixture was shaken and allowed to stand at room temperature for 1 hour. A few drops of methanol were added and after 10 minutes the mixture was diluted to 45 ml. with water. The mixture was extracted with three 15-ml. portions of methylene chloride, the extracts were combined and the solvent was evaporated. The residue was crystallized from 0.1 ml. of methanol to yield 19 mg. of 11-ketoprogesterone (V), m.p. 172–175°, $[\alpha]^{25}D$ +227° (chloroform).

Anal. Calcd. for $C_{21}H_{28}O_3$: C, 76.79; H, 8.59. Found: C, 76.59; H, 8.55.

The various physical constants for V were identical with those of 11-ketoprogesterone.[10] The infrared curves of compound V and authentic 11-ketoprogesterone were also identical.[1]

11α-Acetoxyprogesterone.—To 20 mg. of compound II was added 0.6 ml. of pyridine and 0.6 ml. of acetic anhydride. The mixture was allowed to stand for 16 hours at room temperature and 25 ml. of water was added. After one hour, the mixture was refrigerated to induce crystallization. The crystals which formed were recovered by filtration, washed with water and dried to yield 16.1 mg. of 11α-acetoxyprogesterone VI, m.p. 175–177°, $[\alpha]^{24}D$ +143° (acetone).

Anal. Calcd. for $C_{23}H_{32}O_4$: C, 74.16; H, 8.66. Found: C, 74.33; H, 8.78.

Infrared analysis indicated the absence of a hydroxyl group and the presence of an acetoxy group.

The propionate of 11α-hydroxyprogesterone was prepared with propionic anhydride and pyridine and crystallized twice from ethyl acetate; m.p. 145.5–146°, $[\alpha]^{25}D$ +156° (chloroform).

Anal. Calcd. for $C_{24}H_{34}O_4$: C, 74.58; H, 8.87. Found: C, 74.81; H, 8.74.

The benzoate of 11α-hydroxyprogesterone was prepared with benzoyl chloride and pyridine and crystallized twice from ether; m.p. 181–183°, $[\alpha]^{23}D$ +88° (chloroform).

Anal. Calcd. for $C_{28}H_{34}O_4$: C, 77.38; H, 7.89. Found: C, 77.57; H, 7.94.

The formate of 11α-hydroxyprogesterone was prepared with formic acid and crystallized twice from methanol; m.p. 162–164°, $[\alpha]^{23}D$ +179° (chloroform).

Anal. Calcd. for $C_{22}H_{30}O_4$: C, 73.71; H, 8.44. Found: C, 73.69; H, 8.38.

C. Progesterone Bioconversion by *Rhizopus nigricans* Ehrb. (A.T.C.C. 6227b). Small Scale. 11α-Hydroxyprogesterone (II).—To 12.0 liters of a 24-hour growth of *Rhizopus nigricans* was added 6.0 g. of progesterone in 150 ml. of acetone. After a 24-hour transformation period the beer and mycelium were extracted as previously described, and the extract was concentrated. Crystallization from the methylene chloride sirup was carried out with ether as described to give 5.072 g. (81% yield) of crystalline 11α-hydroxyprogesterone (II), m.p. 165–168°. Four hundred mg. of this product was recrystallized from methanol to give 311 mg., m.p. 167–169°. Two additional recrystallizations from methanol yielded 200 mg., m.p. 166–168°, $[\alpha]^{24}D$ +180° (chloroform).

Anal. Calcd. for $C_{21}H_{30}O_3$: C, 76.32; H, 9.15. Found: C, 76.77; H, 8.92.

This product was identical in every respect to 11α-hydroxyprogesterone obtained from the fermentations of progesterone with *Rhizopus arrhizus*.

Dihydroxyprogesterone (III).—By alumina chromatography of the residue in the mother liquors obtained from the direct crystallization of II, a small amount of III (about 0.5% yield) was obtained; this product was identical to the dihydroxyprogesterone from the fermentations with *Rhizopus arrhizus*. The chromatographic column was similar to that previously described.

Larger Scale. 11α-Hydroxyprogesterone (II).—To a 24-hour growth of *Rhizopus nigricans* in 240 l. of medium, 120 g. of U.S.P. progesterone was added in acetone. After a 30-hour transformation period the beer and mycelium were processed as previously described. Papergram analysis indicated a 90–97% conversion to II. Upon evaporation of the solvent on a steam-bath using a stream of air, 194 g. of a crystalline mass was obtained. Washing eight times with 100-ml. portions of a mixture of one part of methylene chloride and nine parts of ether yielded 102.32 g. of product, m.p. 165–168°, $[\alpha]^{23}D$ +174° (chloroform). Since an equivalent of 1.0 g. of progesterone was removed for testing purposes, this represents a yield of 83%. Papergram analysis indicated the following impurities: 1% of I, 0.8% of III, and 4% of 11α-hydroxyallopregnanedione (IV). The mother liquors yielded an additional 3.78 g. of crystals, m.p. 142–154° and 2.95 g. of crystals, m.p. 131–140°.

11α-Hydroxyallopregnane-3,20-dione (IV).—The compound regarded as IV by papergram analysis was present in only small amounts (0.5–5%). It was isolated from the mother liquors of 11α-hydroxyprogesterone obtained from the fermentation of 1000 g. of progesterone. Two grams (7%) of the mother liquor concentrate, containing about 15% of this steroid, was chromatographed over 50 g. of alumina (acid-treated, water-washed and heated to 120° for 2 hours). The column was developed with 100-ml. portions of solvents in a manner similar to the column previously described. The ether +50% chloroform and the chloroform eluates (689 mg.), which were shown by papergram analysis to be greatly enriched with IV, were combined and rechromatographed over alumina. The benzene +5% ether and the benzene + 10% ether fractions (680 mg.) were shown to contain IV as well as some quantities of 11α-hydroxyprogesterone. One crystalline fraction, no. 5 (362 mg.), which had a m.p. of 178–185°, was shown by infrared studies to be a fairly good sample of IV. This fraction was dissolved in 30 ml. of hot ethyl acetate, filtered and concentrated to 3 ml. Crystals began to form and after 2 hours at room temperature 203 mg. of crystals, m.p. 195–197°, $[\alpha]^{23}D$ + 93° (chloroform) was obtained. Recrystallization and chromatography a second time over alumina as before yielded 66 mg., m.p. 198–200°, $[\alpha]^{23}D$ + 84°. These physical constants as well as infrared absorption were identical with those of an authentic sample of pure 11α-hydroxyallopregnane-3,20-dione (IV) prepared by Dr. A. H. Nathan of our laboratories.[18]

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KALAMAZOO, MICHIGAN

(18) To be published later.